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Reproducibility of early and late asthmatic responses to allergen challenge in a large group of asthmatics

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The specific bronchial provocative test (sBPT) coupled with allergen is used to investigate asthma. Very few studies have examined the reproducibility of responses to allergen challenge. The aim of this study was to measure the reproducibility of $PD_{20}FEV_1$ allergen and late asthmatic response (LAR) in 53 asthmatics and to relate the reproducibility to the time interval between two allergen challenges.

Fifty-three atopic asthmatics performed two allergen challenges not less than 2 and not more than 26 weeks apart. Randomly, 19 subjects were assigned to a *short-interval* group (14–35 days between the two tests) and 34 to a *long-interval* group (40–180 days). In each challenge, the $PD_{20}FEV_1$ was sought for and the maximum % fall in FEV_1 from 3 to 7 h after the allergen challenge was evaluated as a measurement of magnitude of the LAR.

High intraclass correlation coefficients (R_I) were found for both $PD_{20}FEV_1$ ($R_I = 0{\cdot}78$) and LAR ($R_I = 0{\cdot}77$) in all subjects. $PD_{20}FEV_1$ allergen showed a high R_I in the *long-interval* group ($R_I = 0{\cdot}80$), but a low R_I in the *short-interval* group ($R_I = 0{\cdot}63$). In contrast LAR showed a lower R_I in the *long-interval* group ($R_I = 0{\cdot}68$) than in the *short-interval* group ($R_I = 0{\cdot}77$). Moreover, the R_I for $PD_{20}FEV_1$ was particularly low in subjects with a dual pattern to the allergen challenge and a *short interval* between the two allergen challenges.

Our study confirmed that asthmatic responses induced by allergen challenge have a good reproducibility. Moreover, we have demonstrated that the interval between two allergen challenges can determine a change in reproducibility in asthmatic responses induced by allergen challenge.

Key words: reproducibility; allergen early asthmatic response; allergen late asthmatic response.

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Introduction

Inhalation of a specific allergen in sensitized asthmatics can induce an early asthmatic response (EAR), developing 10–30 min after allergen challenge, and a late asthmatic response (LAR), which occurs a few hours (usually 6–8 h) after allergen inhalation (1). LAR resolves more slowly than EAR, and mimics spontaneous asthma attacks (2). Allergen-induced asthmatic responses, especially LAR, can be used to investigate the pathophysiology of asthma (3,4) and to determine bronchial sensitivity to a specific allergen (1). Moreover, allergen challenge is a useful method of evaluating the efficacy of new medications, in the treatment of asthma (1,5).

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Any meaningful interpretation of allergen challenge results should be able to reproduce EAR and LAR. Several studies have examined the reproducibility of responses to allergen challenge (6–13). Generally, the good repeatability of an early response was described either when incremental doses of allergen were inhaled to a provocative and (6,7,11), or when the magnitude of the EAR was compared with a similar allergen dose in both tests (8,9,12,13). Some studies evaluated positive the reproducibility of the magnitude of the LAR (8,9). These data are necessary to calculate sample sizes required to show significant attenuation of both early and late responses, and to identify appropriate uses of allergen challenge (9). However, the relationship of within-subject variability of EAR and LAR to the interval between the two challenges has not yet been published.

The aim of this study was to measure the reproducibility of allergen-induced early and late asthmatic responses in a large sample of asthmatic patients and to relate the reproducibility of EAR and LAR to the time between the two challenges.

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Methods

SUBJECTS

Fifty-three subjects (23 female and 30 male, mean age 25 $\pm$10 years [range 15, 54]) with mild asthma were selected. All were examined in a stable phase of the disease, and had been free of respiratory infections and asthmatic exacerbations during the previous 4 weeks. In a preliminary evaluation, each subject had positive skin prick tests to *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*, Graminaceae or Parietaria (mean wheal diameter, subtracted from negative control, $\geq$5 mm). Forty two subjects were sensitized to house dust mite, 10 to *Graminaceae*, one to *Parietaria officinalis*. Baseline FEV_1 was more than 65% of the predicted normal value in all subjects on all study days. Forty-three subjects indicated the presence of non specific bronchial hyperresponsiveness to methacholine ($PD_{20}FEV_1 < 1.0$ mg).

STUDY DESIGN

Subjects attended for a baseline evaluation, including personal history, a methacholine inhalation challenge test and allergy skin prick tests. Subjects then underwent two allergen inhalation challenge tests, not less than 2 and not more than 26 weeks apart. Although some of them had previously used sodium cromoglycate and inhaled glucocorticosteroids, in the month preceding the study all patients were treated with inhaled salbutamol prn only. Before each challenge, short-acting β_2-agonists were withheld for 12 h. Randomly, in a 1:2 ratio for short *vs*. long-term reproducibility (in blocks of six subjects) 19 subjects were assigned to the group with a short interval (14–35 days) and 34 to the group with a long interval (40–180 days) between the two challenges. Each sought for the dose that provoked a 20% decrease in FEV_1 ($PD_{20}FEV_1$). Subjects whose asthma or respiratory infections had exacerbated or who required steroid treatment during the interval between the two allergen challenges were excluded from the study. Patients sensitive to Graminaceae and/or Parietaria were studied out of the pollen season. This study was approved by the local ethics committee.

SPECIFIC BRONCHIAL PROVOCATIVE TEST WITH ALLERGEN (sBPT)

sBPT was performed with allergens standardized to biologic units (BU). Allergen extract solution was delivered by a DeVilbiss 646 jet nebulizer (DeVilbiss Health Care, Somerset, PA, U.S.A.) using a procedure previously described (14). Lyophilized allergen extract (NeoAbellò, Milano, Italy) was dissolved in saline in order to obtain two working solutions with different concentrations (1 and 10 BU/ml). The nebulizer was filled with 3 ml of diluent (phenol 0.4% in saline) or allergen solution, and connected to a dosimeter (Passerini, Pontedera, Italy) driven by compressed air and activated as patients inhaled. With the nebulizer vent closed, a 20 psi inlet pressure and 1 sec long nebulization, the output was 10 $\pm$1 μl, measured by weighing the nebulizer before and after one discharge. The aerodynamic mass median diameter of the aerosol generated was 1·2 μm (geometric standard deviation, 2·9), measured with a cascade impactor. The output of the nebulizer was regularly checked every 3 months. Each subject wore a nose clip and was instructed to breath via a mouthpiece from functional residual capacity. The nebulizer was filled with 3 ml of allergen solution or diluent control filtered through a 0·2 µm millipore filter. After baseline evaluation, the patient inhaled three puffs of diluent, followed at 10 min intervals by increasing doses of allergen to obtain the following cumulative logarithmic doses, 0·025, 0·05, 0·1, 0·2, 0·4, 0·8, 1·6, and 3·2 BU. FEV_1 was measured 10 min after the end of each allergen dose by means of a water sealed bell spirometer connected to an Olivetti computer (Biomedin, Padova, Italy). The inhalations were continued until FEV_1 fell more than 20% below the post-diluent value or until the last dose of allergen had been administered, and the total dose (TD) of allergen delivered was computed. FEV_1 was then measured at 20, 30 and 60 min, and then hourly for 7 h. A fall in FEV_1 greater than 20% between 10 and 60 min, and between the third and the seventh hour after the challenge was considered to be an early asthmatic response (EAR) and a late asthmatic response (LAR), respectively.

The dose of allergen causing a 20% fall in FEV_1 from a post-diluent value ($PD_{20}FEV_1$ allergen) was derived from the following formula:

$$PD_{20}FEV_1 - anti\log\left(\log(preTD) + \left(\frac{(\log(TD) - \log(preTD)) + (-20 - \Delta FEV_1\%_{preTD})}{(\Delta FEV_1\%_{TD} - \Delta FEV_1\%_{preTD})}\right)\right),$$

where: TD = threshold dose; preTD = previous dose to threshold dose; $\Delta FEV_1\%_{TD}$ = % fall of FEV_1 with respect to baseline at TD; $\Delta FEV_1\%_{preTD}$ = % fall of FEV_1 with respect to preTD; antilog = anti-logarithm.

Subjects were included in the study if a decrease in FEV_1 greater than 20% was observed during the early phase of the first challenge.

STATISTICAL ANALYSIS

Baseline FEV_1 was expressed as a percent of the predicted value. Allergen-induced EAR was calculated as the maximal percent decrease in FEV_1 from baseline within the first hour after allergen, while LAR was evaluated as the maximal percent decrease in FEV_1 between 3 and 7 h after allergen. These values were expressed as means and SE. Logarithmic transformation was used to compare allergen $PD_{20}FEV_1$. T test to compare FEV_1, FEV_1 percentage falls and $\log PD_{20}FEV_1$ was used. A level of probability lower than 5% was considered significant. Reproducibility of $PD_{20}FEV_1$ and LAR after allergen inhalation was plotted according to the method proposed by Bland and Altman (15). Intraclass correlation coefficient was calculated by

two-way ANOVA (16). Graphics of predicting sample sizes were constructed by published power analysis (17).

Results

Fifty-eight subjects performed the first sBPT, of whom 53 also completed the second sBPT.

There was no significant difference between the first and second allergen challenge as regards baseline FEV_1, $PD_{20}FEV_1$ allergen, and $\Delta FEV_1\%$ during LAR, while $\Delta FEV_1\%$ during EAR was significantly greater in second test ($\Delta FEV_1\%$, mean ± SE, −33·6 ± 1·3 *vs.* −37·0 1·4 in the first and second test respectively, $P<0{\cdot}05$) (Table 1).

In all subjects, the reproducibility of $PD_{20}FEV_1$ allergen calculated in the first allergen challenge (BU, geometric mean, 0.174) compared well with that of the second one (0·162 BU) ($R_I = 0{\cdot}78$) (Fig. 1). No significant reproducibility in $\Delta FEV_1\%$ during EAR between the two tests was found ($R_I = 0{\cdot}16$).

Forty-one subjects had a LAR after the first allergen challenge ($\Delta FEV_1\%$, mean ± SE: −28·4% ±2·3), and 45 had a LAR after the second allergen challenge (−27·8% + 2·1). The magnitude of the FEV_1 decrease during the LAR was reproducible ($R_I = 0{\cdot}77$) (Fig. 1).

The patients were randomly grouped into a *short-interval* group of 19 subjects, who performed the second allergen challenge 14–35 days after the first allergen challenge (days, mean: 19·7, and a *long-interval* group of 34 subjects the second test 1–5 months after the first allergen challenge (days, mean: 83·4. The two groups were comparable for age, sex distribution, baseline FEV_1, but there was a greater reactivity to methacholine in the *short-interval* group in comparison with the *long-interval* group (mg, GM: 0.080 *vs* 0·283, $P<0{\cdot}05$). There was no difference between the two groups as regards $PD_{20}FEV_1$ allergen, $\Delta FEV_1\%$ during EAR and LAR (Table 2).

The $PD_{20}FEV_1$ allergen was highly reproducible in the *long interval* group (BU, geometric mean, 0·147 after the first allergen challenge and 0·152 after the second, R_I = 0·80), while the intraclass correlation coefficient was lower in the *short interval* group (0·229 after the first allergen challenge and 0·191 after the second, $R_I = 0{\cdot}63$). In contrast, the magnitude of LAR showed a high R_I in the *short interval* group ($FEV_1\%$, mean ± SE: −24·3% ± 3·3 after the first allergen challenge and −25·9% ± 3·4 after the second $R_I = 0{\cdot}77$) but it was lower in the *long interval* group (−30·3% ± 3·0 after the first allergen challenge and −28·9% ± 2·7 after the second, $R_I = 0{\cdot}68$) (Figs 2 and 3). In an individual analysis, 12 out of 19 patients in the *short interval* group had a LAR in the first sBPT and 13 in the second. On the other hand, 24 out of 34 subjects the *long interval* group had a LAR in the first sBPT and 23 in the second.

Moreover, $PD_{20}FEV_1$ allergen in subjects with EAR alone separated from subjects with the dual response (EAR+LAR), had a high intraclass correlation coefficient in 28 patients with the dual pattern and a *long-interval* between the two tests ($R_I = 0{\cdot}79$), This result was also evident in the two subgroups of subjects with the EAR pattern (six in the *short-interval* group, $R_I = 0{\cdot}77$; six in the *long-interval* group, $R_I = 0{\cdot}92$), while 13 patients with the dual pattern and a *short-interval* between the two allergen challenges had a low intraclass correlation coefficient (R_I = 0·55).

The required sample sizes needed to show a statistically significant attenuation of an expected magnitude in the asthmatic responses to allergen at a given power level are illustrated in Fig. 4. Thus, if a drug was expected to produce a 50% attenuation of the LAR, then less than 10 subjects would be needed to show significance at a power level of 0·80 in a crossover study. If we consider $PD_{20}FEV_1$, then 14 subjects are needed to show a change of one doubling dose at a power level of 0·80.

Discussion

Our study has demonstrated that the time between two allergen challenges in sensitized subjects can influence the repeatability of asthmatic responses. Moreover, our results confirm the good reproducibility of asthmatic responses to allergen in a large group of asthmatics, thus producing further evidence for use of small samples in laboratory studies.

Both asthmatic responses can be influenced by technical and non-technical factors related to the procedure. Technical factors include the characteristics of the allergen extracts, methods of aerosol generation, use of an appropriate dosimeter, and measurements of response (18). Of the non technical factors, including subject characteristics (19), many medications can, at least partially, inhibit

TABLE 1. Characteristics of subjects at baseline evaluation and comparing the first and second allergen challenges

	1st allergen challenge	2nd allergen challenge
FEV_1 baseline (% of predicted, mean ± SEM)	97·6 ± 1·7	97·7 ± 1·8
$PD_{20}FEV_1$ allergen. (BU, GM and range)	0·174 [0·01, 2·84]	0·162 [0·01, 3·02]
EAR ($\Delta FEV_1\%$, mean ± SEM)	−33·6 ± 1·3 *	−37·0 ± 1·4
LAR ($\Delta FEV_1\%$, mean ±SEM)	−28·4 ± 2·3	−27·8 ± 2·1

* = $P<0{\cdot}05$ *vs.* second allergen challenge.
BU = Biologic units; GM = geometric mean.

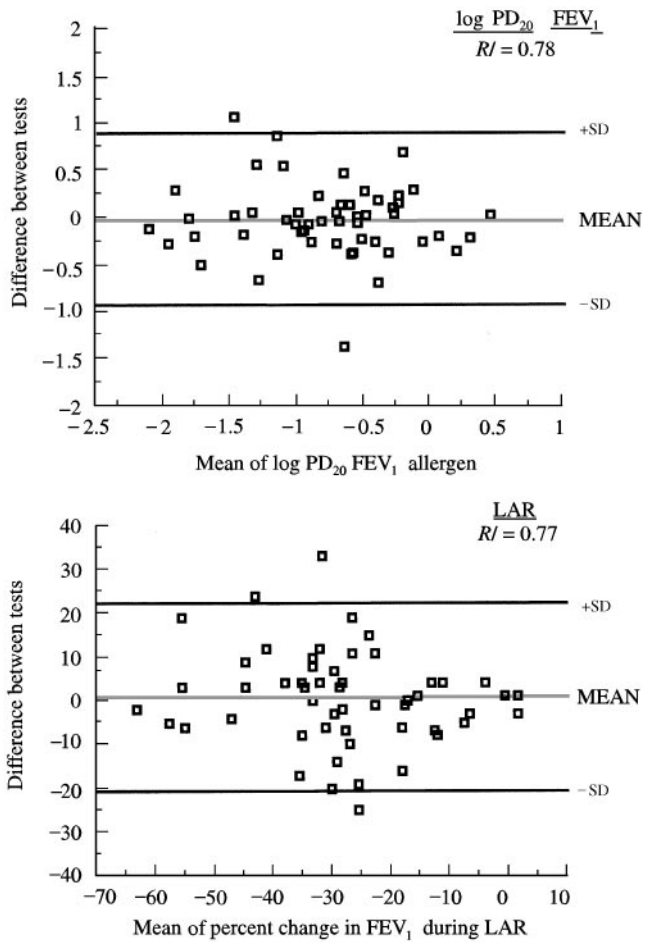

FIG 1. Upper panel. Repeatability of the $PD_{20}FEV_1$ allergen after two allergen challenges performed on two different days in 53 mild asthmatic adults. Lower panel. Repeatability of the magnitude of late asthmatic response (LAR) after two allergen challenges performed on two different days in 53 mild asthmatic adults.

TABLE 2. Comparison between the first and second allergen challenges in the short and 'long-interval' groups of asthmatics

	Short-interval group		Long-interval group	
no.	19		34	
Time interval (days, mean and range)	19·7 [15, 30]*		83·4 [45, 150]	
Age (yr, mean ± SD)	23·7 ± 8·3		25·9 ± 10·6	
Sex (M/F)	11/8		19/15	
$PD_{20}FEV_1$ methacholine (mg, GM and range)	0·080 [0·001, 1·391]*		0·283 [0·283 [0·29, 4·0]	
	1st allergen challenge	2nd allergen challenge	1st allergn challenge	2nd allergen challenge
FEV_1 baseline. (% of pred., mean ± SEM)	102·1 ± 3·1	100·0 ± 3·2	95·3 ± 2·0	96·5 ± 2·3
$PD_{20}FEV_1$ Allergen (BU, GM and range)	0·23 [0·02, 1·48]	0·19 [0·01, 1·08]	0·15 [0·01, 2·84]	0·15 [0·01, 3·02]
EAR (ΔFEV_1%, mean ± SEM)	−33·8 ± 1 ± 8	−33·5 ± 1·7*	−33·4 ± 1·8	−38·9 ± 1·9
LAR (ΔFEV_1%, mean ± SEM)	−24·3 ± 3 ± 3	−25·9 ± 3·4	−30·8 ± 3·0	−28·9 ± 7

* = $P < 0.05$ *vs.* long-interval group.
BU = biologic Units; GM = geometric mean.

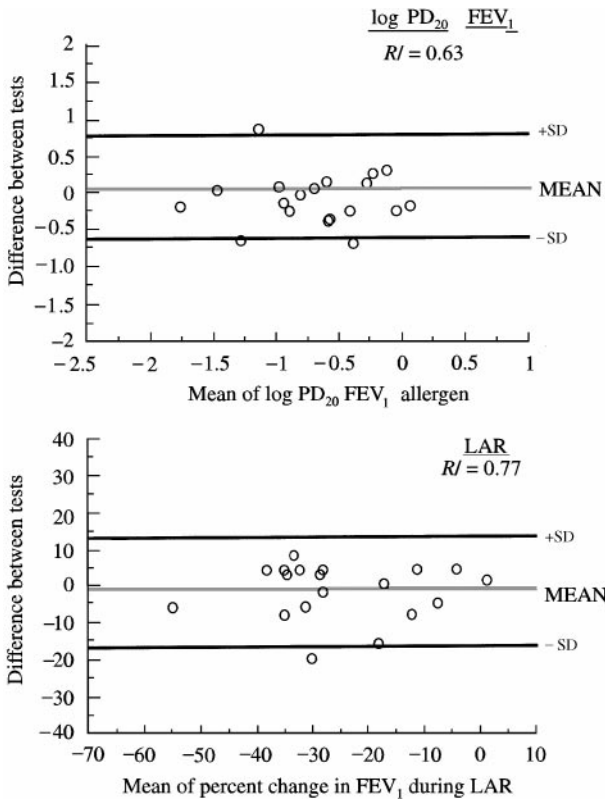

FIG. 2. Upper panel. Repeatability of the $PD_{20}FEV_1$ allergen after two allergen challenges performed on two different days at interval of lesser than 35 days in 19 mild asthmatic adults. Lower panel: Repeatability of the magnitude of the late asthmatic response (LAR) after two allergen challenges performed on two different days at intervals of less than 35 days in 19 mild asthmatic adults.

asthmatic responses to allergen (20), while recent allergen exposure (21) and previous respiratory infections (22,23) may induce an increase of airway inflammation and a change in allergen sensitivity and in the pattern of response.

Few studies examined the reproducibility of an early response to allergen challenge, expressed as a provocative dose (6,7,11). There is some discrepancy in the results. Kopferschmitt-Kubler and co-workers (6) investigated the reproducibility of the early asthmatic response to house dust mite in 14 asthmatics and found a high correlation coefficient for $PD_{20}FEV_1$ after repeating the allergen challenge after an interval of 2-weeks. However, the intraclass correlation coefficient calculated by the same data was low (R_I = 0·31). In our interpretation, this low repeatability is due to the short interval between the two tests. Rosenthal and co-workers (7) performed a 4 day bronchial inhalation challenge to ragweed pollen plethy in 13 seasonal asthmatic patients, and airway conductance was measured in the body pletismograph, calculating the PD_{35}sGaw. Investigators confirmed that the reproducibility was satisfactory. But it is possible to calculate PD_{35}sGaw, a high RI between the 1st and 2nd day (R_I = 0·81), as well as between the 1st and the 3rd day (R_I = 0·83), and a significantly lower R_I between the 1st and the 4th day (R_I = 0·56). This result can be explained by the short interval between allergen challenge tests and consequently by the cumulated influence of previous tests on subsequent tests. On the other hand, Frølund and co-authors (11) studied the reproducibility of EAR in 13 asthmatics sensitized to various allergens within an interval of 14 days with 10-fold increasing concentrations of allergen solutions. They found that $PC_{20}FEV_1$ had a good reproducibility (CoV 8·5%; R_I = 0·99). It is possible that the subjects of that study had a response pattern (e.g. early response alone) to the allergen challenge but that the short interval between the two tests did not influence the $PC_{20}FEV_1$. We have demonstrated in our study that the provocative dose of allergen is significantly changed between two tests only in subjects with a dual pattern.

In our study, a low reproducibility of the magnitude of the EAR, expressed as ΔFEV_1%, was found, similar to other studies (9,24). This is because of the design of the specific bronchial test, performed to obtain an immediate ΔFEV_1% in a narrow range. Consequently, this narrow range of an early fall of FEV_1 prevents a high intraclass coefficient for that variable. In other studies, investigators found good reproducibility of the magnitude of EAR (8,12,13), but they probably had a greater range of response, which improves the calculation of the intraclass correlation coefficient.

Only a few studies have examined the reproducibility of LAR due to allergen challenge. Cockcroft and co-workers found it to be rather low, but calculated it as a coefficient of variation (10). Two studies evaluated the reproducibility of LAR as an intraclass correlation coefficient, with a high R_1 value (8,9). Our study confirms this good repeatability of LAR, but that the repeatability of LAR is less accurate when the interval between the two tests is longer than 30 days.

In this study, we have demonstrated that the duration of time between two allergen challenges in sensitized subjects influences the repeatability of measurements of EAR and LAR.

EAR expressed as $PD_{20}FEV_1$, has a good reproducibility if the interval between the two challenges is longer than 30 days. If the interval is shorter than 30 days, the second $PD_{20}FEV_1$ is less satisfactory than the first. More accurately, we have demonstrated that the shortness of the interval has an effect on the repeatability of the $PD_{20}FEV_1$ allergen if a LAR is present, while the $PD_{20}FEV_1$ allergen is not influenced by the duration of the time interval in subjects with EAR alone. The functional effects of a LAR, as symptoms and/or as non specific bronchial hypereactivity, can persist for a long period after an allergen challenge (2,25,26). Thus, we suggest that, in studies in which a comparison between $PD_{20}FEV_1$ allergen is required, sensitized subjects with an isolated EAR to allergen can be included following allergen challenges. In addition, if the interval between challenges is short, deterioration of the immediate response to allergen in the second test should be minimal. We have demonstrated that LAR shows good reproducibility only when the interval is short, while the repeatability of the magnitude

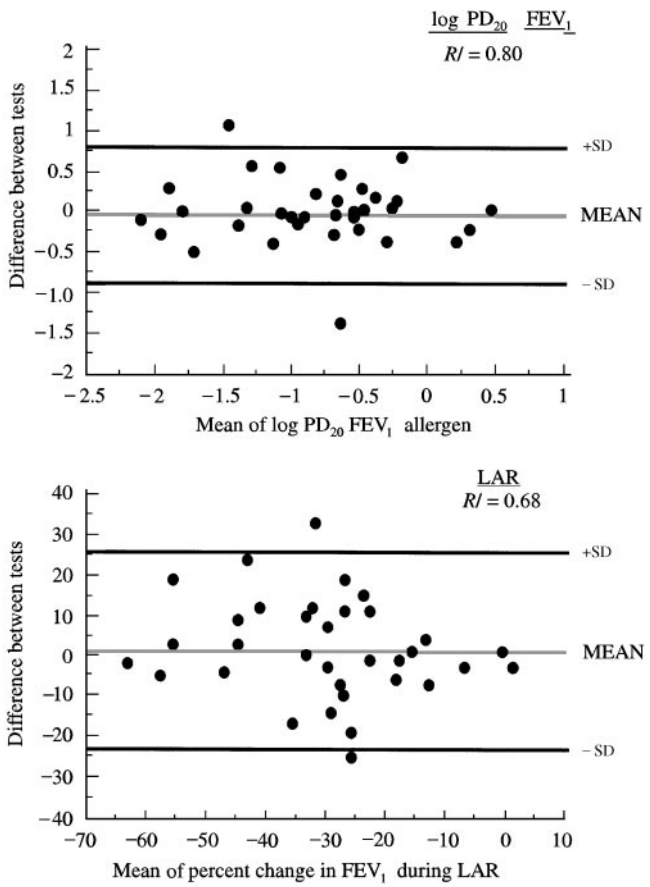

FIG 3. Upper panel. Repeatability of the $PD_{20}FEV_1$ allergen after two allergen challenges performed on two different days at intervals greater than 35 days in 34 mild asthmatic adults. Lower panel. Repeatability of the magnitude of the late asthmatic response (LAR) after two allergen challenges performed on two different days at intervals greater than 35 days in 34 mild asthmatic adults.

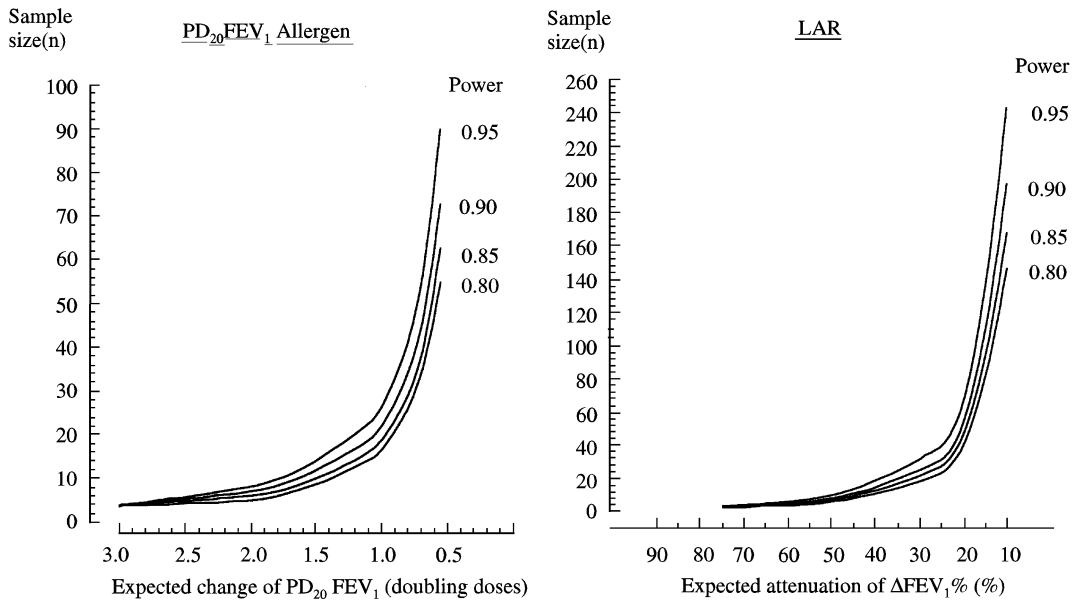

FIG 4. Curves allowing estimation of sample sizes for given power and expected attenuation of $PD_{20}FEV_1$ allergen (right) and maximal decrease in FEV_1 during late asthmatic response (LAR) (left).

of LAR is less accurate when the interval is longer than 30 days. We argue that, with a longer interval, it is easier for new conditions (e.g. exposure to allergen or also airway infections) to alter the magnitude of the LAR, according to experimental data (21–23). Consequently, when measuring the effects of treatment on the LAR, subsequent allergen challenges must be separated by intervals of less than 35 days.

The high reproducibility of the EAR and LAR measurements after allergen challenge allows sample sizes to be used in order to detect changes in asthmatic responses in experimental conditions. These findings support the results of studies in which significant effects of drugs, on both EAR and LAR have been found with sample sizes of about 12 subjects.

In conclusion, our study shows that asthmatic responses induced by allergen challenge are reproducible but that the interval between two allergen challenges is crucial in order to obtain a good reproducibility of EAR and LAR.

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